

Amendments to the Specification:

On page one, under the heading "Related Applications" please amend the first paragraph as follows:

This patent application is a divisional of application serial no. 10/154,890, filed on May 23, 2002, which is a continuation of patent application serial no. 08/108,591, filed November 22, 1993, now U.S. Patent No. 6,395,474, which is a 371 of PCT/EP92/01219 which is a continuation-in-part of the following Danish Patent Applications: No. 986/91, filed May 24, 1991, No. 987/91, filed May 24, 1991 and No. 510/92, filed April 15, 1992. The disclosure of each of the foregoing applications is hereby incorporated by reference in its entirety.

On page 14, lines 1-8 please amend the paragraphs as follows:

Figures 11a and 11b show binding of AcrT₁₀-Lys to dA₁₀- 5'-³²P-labeled oligonucleotide (1) (5'-GATCCA₁₀G) (SEQ ID NO:1) was incubated in the absence or presence of Acr-T₁₀-LysNH₂ and in the absence or presence of oligonucleotide (2) (5'-GATCCT₁₀G) (SEQ ID NO:2) and the samples were analyzed by polyacrylamide gel electrophoresis (PAGE) and autoradiography under "native conditions" (Figure 11A) or under "denaturing conditions" (Figure 11b).

On page 14, lines 9-18 please amend the paragraphs as shown below:

Figures 12a-c show chemical, photochemical and enzymatic probing of dsDNA-Acr-T₁₀-

LysNH₂ complex. Complexes between Acr-T₁₀-LysNH₂ and a ³²P-endlabeled DNA fragment containing a dA₁₀ (SEQ ID NO:3)/dT₁₀ (SEQ ID NO:4) target sequence were probed by affinity photocleavage (Figure 12a, lanes 1-3; Figure 12b, lanes 1-3), photofootprinting (Figure 12a, lanes 5-6), potassium permanganate probing (Figure 12b, lanes 4-6) or probing by staphylococcus nuclease (Figure 12b, lanes 8-10) or by nuclease S₁ (Figure 12c). Either the A-strand (Figure 12a) or the T-strand (Figures 12b,c) was probed.

On page 15, lines 3-7, please amend the paragraph as follows.

Figure 22 shows a graph based on densitometric scanning on PAGE autoradiographs demonstrating the thermal stabilities of PNAs of varying lengths bound to an A₁₀ (SEQ ID NO:3)/T₁₀ (SEQ ID NO:4) double stranded DNA target.

On page 19, lines 8-17 please amend the paragraph as shown below.

Using the S₁-nuclease probing technique, the discrimination of binding of the T₁₀, T₃CT₄(T₉C) & T₂CT₂CT₄(T₈C₂) PNA to the recognition sequences A₁₀ (SEQ ID NO:4), A₅GA₄(A₉G) (SEQ ID NO:5) & A₂GA₂GA₄(A₈G₂) (SEQ ID NO:6) cloned into the *Bam*HI, *Sal*I or *Pst*I site of the plasmid pUC19 was analyzed. The results (Figure 20) show that the three PNAs bind to their respective recognition sequences with the following relative efficiencies: PNA - T₁₀: A₁₀ > A₉G >> A₈G₂, PNA - T₉C: A₉G > A₁₀ ~ A₈G₂, PNA - T₈C₂: A₈G₂ ≥ A₉G >> A₁₀. Thus at 37°C one mismatch out of ten gives reduced efficiency (5-10 times estimated) whereas two mismatches are not accepted.

On page 19, line 33 to page 20, line 5, please amend the paragraph as follows.

The plasmid construct, pT₁₀, contains a dA₁₀ (SEQ ID NO:3)/dT₁₀ (SEQ ID NO:4) tract clones into the *Bam*HI site in pUC19. Thus, cleavage of pT₁₀ with *Bam*HI and *Pvu*II results in two small DNA fragments of 211 and 111 bp, respectively. In the presence of PNA-T₁₀, a 336 bp fragment is obtained corresponding to cleavage only by *Pvu*II (Figure 23). Thus cleavage by *Bam*HI is inhibited by PNA bound proximal to the restriction enzyme site. The results also show that the PNA-dsDNA complex can be formed in 100% yield. Similar results were obtained using the pT8C2 plasmid and PNA-T8C2.

On page 20, lines 13-19, please amend the paragraph as shown below.

The sequence-specific recognition of dsDNA is illustrated by binding of a PNA, consisting of 10 thymine substituted 2-aminoethylglycyl units, which C-terminates in a lysine amide and N-terminates in a complex 9-aminocridine ligand (9-Acr¹-(Taeg)₁₀-Lys-NH₂, Figure 11a, 11b) to a dA₁₀ (SEQ ID NO:3)/dT₁₀ (SEQ ID NO:4) target sequence. The target is contained in a 248 bp ³²P-end-labelled DNA-fragment.

On page 20, lines 22-27, please amend the paragraph as shown below.

1) The 9-Acr¹ ligand (Figure 5), which is equipped with a 4-nitrobenzamido group to ensures cleavage of DNA upon irradiation, is expected only to cleave DNA in close proximity to its binding

site. Upon irradiation of the PNA with the above 248 bp DNA fragment, selective cleavage at the dA₁₀ (SEQ ID NO:3)/dT₁₀ (SEQ ID NO:4) sequence is observed (Figure 3a). A₁₀ (SEQ ID NO:3)/T₁₀ (SEQ ID NO:4) sequence is observed (Figure 3a).

On page 21, lines 3-8, please amend the paragraph as shown below.

4) In yet another type of experiment, the well-known high susceptibility of single strand thymine ligands (as opposed to double strand thymine ligands) towards potassium permanganate oxidation was employed. Oxidation of the 248 bp in the presence of the reagent showed only oxidation of the T₁₀-strand (SEQ ID NO:4) of the target (Figure 3b).

On page 21, lines 9-14, please amend the paragraph as shown below.

5) In a similar type of demonstration, the single strand specificity of S₁ nuclease clearly showed that only the T₁₀-strand (SEQ ID NO:4) of the target was attacked (Figure 3d).

On page 21, lines 3-8, please amend the paragraph as follows.

The very efficient binding of (Taeg)₁₀, (Taeg)₁₀-Lys-NH₂ and Acr¹-(Taeg)₁₀-Lys-NH₂ (Figures 11a, 11b) to the corresponding dA₁₀ (SEQ ID NO:3) was furthermore illustrated in two ways:

On page 21, lines 15-24, please amend the paragraph as shown below.

1. Ligand-oligonucleotide complexes will migrate slower than the naked oligonucleotide

upon electrophoresis in polyacrylamide gels. Consequently, such experiments were performed with $\text{Acr}^1\text{-(Taeg)}_{10}\text{-Lys-NH}_2$ and ^{32}P -end-labelled dA_{10} (SEQ ID NO:3). This showed retarded migration under conditions where a normal dA_{10} (SEQ ID NO:3)/ dT_{10} (SEQ ID NO:4) duplex is stable, as well as under conditions where such a duplex is unstable (denaturing gel). A control experiment was performed with a mixture of $\text{Acr}^1\text{-(Taeg)}_{10}\text{-Lys-NH}_2$ and ^{32}P -end-labelled dT_{10} (SEQ ID NO:4) which showed no retardation under the above conditions.

On page 22, lines 12-26, please amend the paragraph as follows.

List of oligodeoxyribonucleotides:

1. 5'-AAA-AAA-AA
2. 5'-AAA-AAA-AAA-A (SEQ ID NO: 3)
3. 5'-TTT-TTT-TTT-T (SEQ ID NO: 4)
4. 5'-AAA-AAG-AAA-A (SEQ ID NO: 9)
5. 5'-AAG-AAG-AAA-A (SEQ ID NO: 10)
6. 5'-AAA-AGA-AAA-A (SEQ ID NO: 11)
7. 5'-AAA-AGA-AGA-A (SEQ ID NO: 12)
8. 5'-TTT-TCT-TTT-T (SEQ ID NO: 13)
9. 5'-TTT-TCT-TCT-T (SEQ ID NO: 14)
10. 5'-TTT-TTC-TTT-T (SEQ ID NO: 15)
11. 5'-TTT-TTC-TTC-T (SEQ ID NO: 16)
12. 5'-TTC-TTC-TTT-T (SEQ ID NO: 17)
13. 5'-TTT-TTT-TTT-TTT (SEQ ID NO: 18)
14. 5'-AAA-AAA-AAA-AAA-AAA (SEQ ID NO: 19)

On page 68, lines 15-30, please amend the paragraph as follows.

Binding of $\text{Acr}^1\text{-(Taeg)}_{10}\text{-Lys-NH}_2$ to dA_{10} (SEQ ID NO:3) (Figure IIa)

$\text{Acr}^1\text{-(Taeg)}_{10}\text{-Lys}$ (100 ng) was incubated for 15 min at room temperature with 50 cps $5'\text{-}[^{32}\text{P}]\text{-end-labelled oligonucleotide [d(GATCCA}_{10}\text{G)]}$ in 20 μl TE buffer (10 mM Tris-HCl, 1

mM EDTA, pH 7.4). The sample was cooled in ice (15 min) and analyzed by gel electrophoresis in polyacrylamide (PAGE). To 10 μ l of the sample was added 2 μ l 50% glycerol, 5 TBE (TBE - 90 mM Tris-borate, 1 mM EDTA, pH 8.3), and the sample was analysed by PAGE (15% acrylamide, 0.5% bisacrylamide) in TBE buffer at 4°C. A 10 μ l portion of the sample was lyophilized and redissolved in 10 μ l 80% formamide, 1 TBE, heated to 90°C (5 min), and analyzed by urea/PAGE (15% acrylamide, 0.5% bisacrylamide, 7 M urea) in TBE. [32 P]-containing DNA bands were visualized by autoradiography using intensifying screens and Agfa Curix RPI X-ray films exposed at -80°C for 2 h.

On page 69, lines 3-24, please amend the paragraph as shown below.

A dA₁₀ (SEQ ID NO:3)-dT₁₀ (SEQ ID NO:4) target sequence contained within a plasmid DNA sequence was constructed by cloning of two oligonucleotides (d(GATCCA₁₀G) (SEQ ID NO:1) + d(GATCCT₁₀G) (SEQ ID NO:2)) into the *Bam*HI restriction enzyme site of pUC19 using the Escherichia coli JM101 strain by standard techniques (Maniatis et al., 1986). The desired plasmid (designated pT₁₀) was isolated from one of the resulting clones and purified by the alkaline extraction procedure and CsCl centrifugation (Maniatis et al., 1986). A 3' - [32 P] -end-labelled DNA fragment of 248 bp containing the dA₁₀ (SEQ ID NO:3) /dT₁₀ (SEQ ID NO:4) target sequence was obtained by cleaving the pT10 DNA with restriction enzymes *Eco*RI and *Pvu*II, labelling of the cleaved DNA with α [32 P]-dATP (4000 Ci/mmol, Amersham) using the Klenow fragment of *E. coli* DNA polymerase (Boehringer Mannheim), and purifying the 248 bp DNA fragment by PAGE (5% acrylamide, 0.06% bisacrylamide, TBE buffer). This DNA fragment was obtained with [32 P]-end-labelling at the 5'-end by treating the EcoRI-cleaved pT10 plasmid with bacterial alkaline phosphatase (Boehringer Mannheim), purifying the plasmid DNA by gel electrophoresis in low melting agarose, and labelling with γ [32 P] ATP and polynucleotide kinase. Following treatment with *Pvu*II, the 248 bp DNA fragment was purified as above.

On page 96, lines 10-28, please amend the table as follows:

Hybridization experiments with the PNA-oligomer H-T₄C₂TCTC-LysNH₂ were performed as follows:

Row	Hybridized With	pH	Tm	§
1	5'-(dA) ₄ (dG) ₂ (dA)(dG)(dA)(dG) (SEQ ID NO: 20)	7.2	55.5	2:1
2	5'-(dA) ₄ (dG) ₂ (dA)(dG)(dA)(dG) (SEQ ID NO: 20)	9.0	26.0	2:1
3	5'-(dA) ₄ (dG) ₂ (dA)(dG)(dA)(dG) (SEQ ID NO: 20)	5.0	88.5	2:1
4	5'-(dG)(dA)(dG)(dA)(dG) ₂ (dA) ₄ (SEQ ID NO: 21)	7.2	38.0	2:1
5	5'-(dG)(dA)(dG)(dA)(dG) ₂ (dA) ₄ (SEQ ID NO: 21)	9.0	31.5	-
6	5'-(dG)(dA)(dG)(dA)(dG) ₂ (dA) ₄ (SEQ ID NO: 21)	5.0	52.5	-
7	5'-(dA) ₄ (dG)(dT)(dA)(dG)(dA)(dG) (SEQ ID NO: 22)	7.2	39.0	-
8	5'-(dA) ₄ (dG)(dT)(dA)(dG)(dA)(dG) (SEQ ID NO: 22)	9.0	<20	-
9	5'-(dA) ₄ (dG)(dT)(dA)(dG)(dA)(dG) (SEQ ID NO: 22)	5.0	51.5	-
10	5'-(dA) ₄ (dG) ₂ (dT)(dG)(dA)(dG) (SEQ ID NO: 23)	7.2	31.5	-
11	5'-(dA) ₄ (dG) ₂ (dT)(dG)(dA)(dG) (SEQ ID NO: 23)	5.0	50.5	-
12	5'-(dG)(dA)(dG)(dA)dT(dG)(dA) ₄ (SEQ ID NO: 24)	7.2	24.5	-
14	5'-(dG)(dA)(dG)(dA)dT(dG)(dA) ₄ (SEQ ID NO: 24)	9.0	<20	-
14	5'-(dG)(dA)(dG)(dA)dT(dG)(dA) ₄ (SEQ ID NO: 24)	5.0	57.0	-
15	5'-(dG)(dA)(dG)(dT)(dG) ₂ (dA) ₄ (SEQ ID NO: 25)	7.2	25.0	-
16	5'-(dG)(dA)(dG)(dT)(dG) ₂ (dA) ₄ (SEQ ID NO: 25)	5.0	39.5	-
			52.0	

On page 97, lines 18-27 as shown below.

The results of hybridization experiments with H-T₅GT₄-LysNH₂ to were performed as

follows:

Row	Deoxyoligonucleotide	T _m
1	5'-(dA) ₅ (dA)(dA) ₄ -3' (SEQ ID NO: 3)	55.0
2	5'-(dA) ₅ (dG)(dA) ₄ -3' (SEQ ID NO: 9)	47.0
3	5'-(dA) ₅ (dG)(dA) ₄ -3' (SEQ ID NO: 9)	56.5
4	5'-(dA) ₅ (dT)(dA) ₄ -3' (SEQ ID NO: 26)	46.5
5	5'-(dA) ₄ (dG)(dA) ₅ -3' (SEQ ID NO: 11)	48.5
6	5'-(dA) ₄ (dC)(dA) ₅ -3' (SEQ ID NO: 27)	55.5
7	5'-(dA) ₄ (dT)(dA) ₅ -3' (SEQ ID NO: 28)	47.0

On page 98, lines 18-24, please amend the table as shown below.

PNA	DNA	T _m
H-T ₁₀ -LysNH ₂	(dA) ₁₀ (SEQ ID NO: 3)	73 °C
H-T ₄ (βT)T ₅ -LysNH ₂	(dA) ₁₀ (SEQ ID NO: 3)	57 °C
H-T ₄ (βT)T ₅ -LysNH ₂	(dA) ₄ (dG)(dA) ₅ (SEQ ID NO: 11)	47 °C
4	(dA) ₄ (dT)(dA) ₅ (SEQ ID NO: 28)	49 °C
5	(dA) ₄ (dT)(dA) ₅ (SEQ ID NO: 28)	47 °C

On page 99, lines 15-26, please amend the table as follows.

PNA	DNA	T _m
H-T ₁₀ -LysNH ₂	(dA) ₁₀ (SEQ ID NO: 3)	73°C
H-T ₄ (Ac)T ₅ -LysNH ₂	(dA) ₁₀ (SEQ ID NO: 3)	49°C
H-T ₄ (Ac)T ₅ -LysNH ₂	(dA) ₄ (dG)(dA) ₅ (SEQ ID NO: 11)	37°C
H-T ₄ (Ac)T ₅ -LysNH ₂	(dA) ₄ (dC)(dA) ₅ (SEQ ID NO: 28)	41 °C
H-T ₄ (Ac) T ₅ -LysNH ₂	(dA) ₄ (dT) (dA) ₅ (SEQ ID NO: 28)	41 °C
H-T ₄ (Ac) T ₅ -LysNH ₂	(dA) ₅ (dG) (dA) ₄ (SEQ ID NO: 9)	36 °C
H-T ₄ (Ac) T ₅ -LysNH ₂	(dA) ₅ (dC) (dA) ₄ (SEQ ID NO: 29)	40 °C
H-T ₄ (Ac) T ₅ -LysNH ₂	(dA) ₅ (dT) (dA) ₄ (SEQ ID NO: 26)	40 °C

On page 100, lines 15-23, please amend the paragraph as follows.

The target plasmids were prepared by cloning of the appropriate oligonucleotides into pUC19. Target A₁₀: oligonucleotides GATCCA₁₀G (SEQ ID NO: 1) & GATCCT₁₀G (SEQ ID NO: 2) cloned into the *Bam*HI site (plasmid designated pT10). Target A₅GA₄: oligonucleotides TCGACT₄CT₅G (SEQ ID NO: 30) & TCGACA₅GA₄G (SEQ ID NO: 31) cloned into the *Sal*I site (plasmid pT9C). Target A₂GA₂GA₄: oligonucleotides GA₂GA₂GA₄TGCA (SEQ ID NO: 32) & GT₄CT₂CT₂CTGCA (SEQ ID NO: 33) into the *Pst*I site (plasmid pT8C2). The positions of the targets in the gel are indicated by bars to the left. A/G is an A+G sequence ladder of target P10.

On page 101, lines 20-28, please amend the paragraph as shown below.

A mixture of 200 cps ³²P-pT₁₀ fragment, 0.5 µg calf thymus DNA and 300 ng of the desired PNA (either T₁₀-LysNH₂, T₈-LysNH₂ or T₆-LysNH₂) was incubated in 100 µl 200 mM NaCl, 50 mM Na-acetate, pH 4.5, 1 mM ZnSO₄ for 60 min at 37 °C. A 2 µg portion of oligonucleotide CATCCA₁₀G (SEQ ID NO:3) was added and each sample 25 was heated for 10 min at the

temperature indicated, cooled in ice for 10 min and warmed to 20°C. A 50 U portion of S₁ nuclease was added and the samples treated and analyzed and the results quantified.

On page 101, line 32 to page 102, line 14, please amend the paragraph as shown below.

A mixture of 100 ng plasmid DNA (cleaved with restriction enzyme *PvuII* (see below) and 100 ng of PNA in 15 µl 10 µM Tris-HCl, 1mM EDTA, pH 7.4 was incubated at 37 °C for 60 min. Subsequently, 4 µl 5 x concentrated buffer (0.2 M Tris-HCl (pH 8.0), 40 mM MgCl₂, 10 mM spermidine, 125 mM NaCl) were mixed with 1 µl NTP-mix (10 mM ATP, 10 mM CTP, 10 mM GTP, 1 mM UTP, 0.1 µCi/µl ³²P-UTP, 5 mM DTT, 2 µg/ml tRNA, 1 µg/ml heparin) and 3 units RNA polymerase. Incubation was continued for 10 min at 37°C. The RNA was then precipitated by addition of 60 µl 2% postassium acetate in 96% ethanol at -20°C and analyzed by electrophoresis in 8% polyacrylamide sequencing gels. RNA transcripts were visualized by autoradiography. The following plasmids were used: pT8C2-KS/pA8G2-KS: oligonucleotides GA₂GA₂GA₄GTGAC (SEQ ID NO: 34) & GT₄CT₂CT₂CTGCA (SEQ ID NO: 33) cloned into the *PstI* site of pBluescript-KS⁺; pT10-KS/pA10-KS (both orientations of the insert were obtained). pT10UV5: oligonucleotides GATCCA₁₀G (SEQ ID NO: 1 & GATCCT₁₀G (SEQ ID NO: 2) cloned into the *BamHI* site of a pUC18 derivative in which the lac UV5 *E.coli* promoter had been cloned into the *EcoRI* site (Jeppesen, *et al.*, *Nucleic Acids Res.*, **1988**, 16, 9545).

On page 107, lines 17-25, please amend the table as shown below.

The title compound hybridized with the following oligonucleotides:

Oligodeoxynucleotide	pH	T _m (°C)
5'-AAT AGT AGT G-3' (SEQ ID NO: 35)	5	31.5†
5'-ATT AGT AGT G-3' (SEQ ID NO:36)	7.2	28.5†

5'-AAT AGT AGT G-3' (SEQ ID NO:36)	9	28.0†
5'-GTG ATG ATA A-3' (SEQ ID NO:37)	7.2	30.5
5'-GTG ATG ATA A-3' (SEQ ID NO:37)	9	28.0

†Low hypochromicity

On page 111, lines 3-10, please amend the specification as shown below.

Hybridization properties of H-[Taeg]₁-[proT]₁-[Taeg]₅Lys-NH₂

Oligodeoxynucleotide	T _m (°C)
5'-AAA AAA AAA A (SEQ ID NO: 3)	53.5
5'-AAA AGA AAA A (SEQ ID NO: 11)	44.0
5'-AAA AAG AAA A (SEQ ID NO: 9)	43.5
5'-AAA ACA AAA A (SEQ ID NO: 27)	46.5
5'-AAA AAC AAA A (SEQ ID NO: 29)	46.5
5'-AAA ATA AAA A (SEQ ID NO: 28)	46.5
5'-AAA AAT AAA A (SEQ ID NO: 26)	46.0

On page 112, lines 5-12, please amend the specification as shown below.

Hybridization properties of H-T₄bCT₅-Lys-NH₂

Oligodeoxynucleotide	T _m (°C)
5'-AAA AAA AAA A (SEQ ID NO: 3)	43.5
5'-AAA AGA AAA A (SEQ ID NO: 11)	58.0

5'-AAA AAG AAA A (SEQ ID NO: 9)	60.0
5'-AAA ACA AAA A (SEQ ID NO: 27)	34.5
5'-AAA AAC AAA A (SEQ ID NO: 29)	34.5
5'-AAA ATA AAA A (SEQ ID NO: 28)	34.0
5'-AAA AAT AAA A (SEQ ID NO: 26)	36.0

On page 113, lines 3-10, please amend the specification as shown below.

Hybridization properties of H-T₄AT₅-LysNH₂

Oligodeoxynucleotide	T _m (°C)
5'-AAA AAA AAA A (SEQ ID NO: 3)	59.5
5'-AAA AGA AAA A (SEQ ID NO: 11)	45.0
5'-AAA AAG AAA A (SEQ ID NO: 9)	45.5
5'-AAA ACA AAA A (SEQ ID NO: 27)	48.0
5'-AAA AAC AAA A (SEQ ID NO: 29)	48.0
5'-AAA ATA AAA A (SEQ ID NO: 28)	52.0
5'-AAA AAT AAA A (SEQ ID NO: 26)	52.5

On page 114, lines 2-5, please amend the specification as follows.

Hybridization properties of crude (approx. 50%) H-T₄G₂TGTG-LysNH₂

Oligodeoxynucleotide	T _m
5'-A ₄ C ₅ ACAC (SEQ ID NO:38)	38

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5'-CACAC ₂ A ₄ (SEQ ID NO: 39)	55
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